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DIGESTIVE ENZYMES OF SUGARCANE PINK BORER, *SESAMIA INFERENS* WALKER (LEPIDOPTERA)

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ABSTRACT

In the salivary glands of *Sesamia inferens*, maltase, sucrase, trehalase, -fructosidase, aminotripeptidase, leucine aminopeptidase, prolinase, glycyl-L-leucine dipeptidase were present. In the gut (tissues and contents of different regions studied separately) in addition to these, melezitase, -glucosidase, -galactosidase, trypsin, glycyl-glycine dipeptidase were also present.

The significance of the synthesis of oligosaccharides in the gut is to avoid hyperglycaemia.

It has been conclusively proved that there are four distinct substrate-specific -glucosidases; and that raffinose is not hydrolyzed by sucrase in *S. inferens*.

INTRODUCTION

Digestion is a process of splitting unabsorbable macromolecules of food by digestive enzymes into utilizable and absorbable molecules by addition of water. Thus the study of the digestive enzymes forms an important base for the study of digestive physiology. The present paper reports the digestive enzymes of salivary glands, and tissue and contents of the different regions of gut of *Sesamia inferens*, a sugarcane borer, and clarifies the ambiguity regarding the oligosaccharide synthesis and glycosidase specificity.

MATERIALS AND METHODS

The field-collected larvae of different ages were reared on sliced sugarcane at 32°C in 6″ glass petri dishes; their diet was changed daily. The last instar larvae were dissected after immobilizing by chilling at —10°C for about 10 min. The salivary glands and the gut were taken out and rinsed quickly with distilled water. The contents of fore-, mid-, and hind-gut

were separately collected in distilled water by tearing the wall which was washed to remove the adhering contents. The salivary glands and the gut wall of each region were homogenized separately. These homogenates and the contents were centrifuged at about 3000 x g for 15 min, and the volume of each supernatant was brought to 1.0 ml by adding distilled water. These supernatants were used as the enzyme source. The reaction mixture contained 0.5 ml of buffer (pH 6.7, 8.2, and 7.0 respectively in case of the enzymes of salivary gland and tissue and contents of foregut, tissue and contents of midgut, and tissue and contents of hindgut); 0.5 ml of 1% substrate; 0.5 ml of enzyme extract and a few drops of toluene. In control mixture the denatured enzyme was taken. Four larvae were used for one test and the presence of the enzymes was detected as described by Agarwal (1975a).

RESULTS

Table 1 shows the qualitative and comparative quantitative presence of different enzymes. In the salivary glands were present three -glucosidases (maltase, sucrase and trehalase), -fructosidase, aminotripeptidase, leucine aminopeptidase, prolinase and glycyl-L-leucine dipeptidase.

In the foregut tissue the activity of only trehalase was detected although the foregut contents showed the presence of all the enzymes detected in the salivary glands.

In the midgut tissue were present three -glucosidases (maltase, melezitase and trehalase), -glucosidase, -galactosidase, trypsin like enzyme, aminotripeptidase, leucine aminopeptidase, prolinase and glycyl-glycine dipeptidase; while in some experiments a very weak amylase activity was also detected. But in the midgut contents were present all the enzymes of its tissue, and sucrase, -fructosidase and glycyl-L-leucine dipeptidase in addition.

In the hindgut tissue were detected only trehalase, a trypsin-like enzyme, leucine aminopeptidase and prolinase; while its contents showed the activity of all these, and sucrase, -glucosidase, aminotripeptidase, glycyl-L-leucine dipeptidase and glycyl-glycine dipeptidase in addition.

When the larvae fed sugarcane having a high percentage of sucrose, an oligosaccharide not present in sugarcane was detected both in the gut contents and excreta. But when they were fed sugarcane having a low percentage of sucrose, this oligo-

saccharide was neither detected in the gut contents nor in the excreta. The same phenomenon was observed *in vitro* when high and low concentrations of sucrose were used as substrate.

When raffinose was hydrolyzed, fructose and melibiose were produced as the end products.

DISCUSSION

In considering the carbohydrates available to *S. inferens* larvae feeding on sugarcane, sucrose is of major nutritional value for them, although glucose and fructose are also present in sugarcane. Maltose, melezitose, trehalose, cellobiose, lactose and raffinose can be utilized by the larvae as is reflected by its available enzyme equipment. Among the carbohydrases, sucrase may therefore be considered as the obligatory enzyme and the other carbohydrases as facultative, as long as the larvae feed on sugarcane. The high sucrase activity in the gut of the larvae and abundance of sucrose in the sugarcane supports the view of House (1965), Wigglesworth (1965) and Dadd (1970) that when there is high activity of any enzyme in the gut of an insect, its diet must contain the corresponding substrate in abundance.

The formation of oligosaccharides in the gut (Duspiva, 1953; Srivastava and Auclair, 1962; Srivastava, 1966; Yang and Davis, 1968; Ishaaya and Swirski, 1970; and Takanona and Hori, 1974) and their excretion (Duspiva, 1953; and Srivastava and Auclair, 1962) have been already reported. Duspiva (1953), Srivastava and Auclair (1962), Yang and Davis (1968) and Ishaaya and Swirski (1970) are of opinion that oligosaccharides are produced by the action of invertase on sucrose. It has been further demonstrated that oligosaccharides can also be synthesized in the presence of melezitose and melibiose (Srivastava, 1966), and maltose (Takanona and Hori, 1974). Gilmour (1961) and Dadd (1970) believed that the synthesis of oligosaccharides is due to transglycolysation reaction of -glucosidases. On this basis Gilmour (1961) regarded invertase as a sugar-transferring enzyme also, and thus recognized two types of functionally different (Dadd, 1970) -glucosidases in insects. In *S. inferens* the production of oligosaccharide is due to reverse catalysis by some glycosidase/s when glucose alone or glucose and fructose both are produced or are present in high concentrations in the gut, as has been pointed out in *Chilotraea infuscatellus* (Agarwal, 1976). Further, the chromatograms of Srivastava and Auclair (1962) also support these observations although their conclusions were different. The oligosaccharide synthesis takes place in an insect as an important measure to limit the diffusion

(absorption) of monosaccharides in the gut when they are in excess and are not needed by the insect. In this manner, hyperglycaemia is avoided in the insect and the oligosaccharide is ultimately excreted. This strategy enables an insect to feed on a variety of diets having varied percentages of utilizable carbohydrates, and therefore the borer continues feeding on sugarcane throughout the year during which period the percentage of sucrose varies widely in sugarcane.

The cane juice contains alanine, -aminobutyric acid, aspertic acid, aspergine, glutamic acid, glutamine, glycine, leucine, lysine, proline, serine and valine (Bhattacharya and Mukherjee, 1953). Besides these, arginine, histidine and isoleucine are present in the proteins of sugarcane (Singh and Singh, 1964). Thus proteases must be very important, in order to release these amino acids from the proteins. In this manner only five essential amino acids (Gilmour, 1965; House, 1965; Wigglesworth, 1965; and Dadd, 1970) are available to the borer in the diet. Methionine, phenylalanine, threonine and tryptophan may be regarded as dispensable essential amino acids and if necessary may be obtained by the transaminase system/s possibly present in the borer.

A few enzymes have been detected in the tissue of the foregut and hindgut of *S. inferens*. Evans and Payne (1964) are of opinion that their presence in these tissues does not mean that they are secreted in the respective lumens. The presence of the enzymes in the foregut contents suggests that the digestion of food starts there or rather as soon as the salivary secretion is mixed with the food-stuff. Although the midgut secretes a large variety of enzymes, the most interesting feature is that the sucrase, the most important carbohydrase, is not secreted but reaches there along with the saliva mixed with the food. By observing the proportionate hydrolysis of the substrates in the fore-, mid- and hind-gut, it becomes evident that the major part of digestion occurs in the midgut. The presence of trehalase in the lumen of the intestine of *S. inferens* is a necessary corollary to the mode of sugar absorption in insects, to prevent loss of trehalose (a reserve carbohydrate) by diffusion as explained by Wyatt (1967).

Day and Waterhouse (1953) have pointed out that the ultimate fate of the enzymes is unknown. In *S. inferens* the presence of some enzymes in the midgut and their simultaneous absence in the hindgut suggests that they are either denatured or digested or self-hydrolysed in the posterior part of the midgut.

Similarly, the presence of some enzymes in the hindgut contents but their absence in its tissue and the excreta also suggests their denaturing, digestion or self-hydrolysis in the posterior part of the hindgut. Some enzymes were detected both in the tissue and contents of the hindgut, their absence or low activity in the excreta may be due to their absorption in the hindgut (Agarwal, 1975b). All this appears to be some kind of economical measure to retain as much enzyme protein as possible.

Hopkins (1932) suggested a high specificity of enzymes while Weidenhagen (1932) simultaneously postulated that a general -glucosidase is responsible for the hydrolysis of all the -glucosides. Weidenhagen's hypothesis continued to prevail till very recently (Fraenkel, 1940; Evans, 1956; Evans and Payne, 1960; Banks, 1963; Evans and Payne, 1964; Zoch, 1965; Khan and Ford, 1967; Dadd, 1970; and Takanona and Hori, 1974) although Pigman (1946), Gottschalk (1950), Neuberg and Mandl (1950), and Gilmour (1961) doubted the hydrolysis of all -glucosides by a single enzyme.

A number of workers (Swingle, 1925; Wigglesworth, 1927; Shinoda, 1930; Babers and Woke, 1937; Hopf, 1938; Parkin, 1940; Ricou, 1958; Saxena, 1958; Rastogi and Dutta Gupta, 1962; and Mathur and Thakar, 1969) showed the presence of two separate -glucosidases: sucrase (invertase or sacchrase) and maltase on the basis of two different substrates. SooHoo and Dudzinski (1967) doubted the hydrolysis of maltose and sucrose by one enzyme.

Krishna (1958) recognized two types of invertases: one attacking on the fructose moiety of the molecule of raffinose and sucrose, while the other attacking glucose moiety of melezitose and sucrose as suggested by Fraenkel (1940). This view prevailed (Evans, 1956; Khan and Ford, 1962; Krishna and Saxena, 1962; Evans and Payne, 1964; Khan and Ford, 1967; and SooHoo and Dudzinski, 1967) till Dadd (1970) doubted the hydrolysis of both raffinose and sucrose by -fructosidase. Banks (1963) and Dadd (1970) believed that the hydrolysis of melezitose and sucrose takes place by the same enzyme.

Evans and Payne (1960) have also suggested that there may be a secretion of more than a single -glucosidase in the desert locust. Davis (1963), although not refuting the presence of a generalized -glucosidase, stressed on the possibility of maltase, sucrase and trehalase separately. Later on, Evans and Payne (1964) suggested two kinds of carbohydrase specificity. Khan

and Ford (1967) also proposed a possibility of " -glucosidase" actually consisting of a complex of enzymes, each splitting the -glucosidic linkages in different substrates. Agarwal (1975a) showed the presence of four separate -glucosidases on the basis of four different substrates.

This kind of confusion continued probably because four distinct situations were not detected in any insect. In *S. inferens* the four situations are absolutely distinct (Table 2), and therefore, the present observations clearly establish the presence of four different -glucosidases in the gut of this insect and each one of them is substrate-specific.

In *S. inferens,* raffinose is not hydrolyzed by sucrase, as is evident by the presence of sucrase and absence of -fructosidase in the hindgut contents. The presence of -fructosidase is established due to the hydrolysis of raffinose and absence of -galactosidase in *S. inferens,* although many workers have suggested that -galactosidase may hydrolyse raffinose also (Agarwal, 1976). In *S. inferens* melezitose is neither hydrolyzed by -fructosidase nor by sucrase; -fructosidase attacks only between fructose and glucose/melibiose* moieties of raffinose**.

The presence of a wide variety of enzymes in the gut of the larvae which are not normally required by it may ensure its capacity to adapt to adverse or diverse nutritional conditions, thus enabling the borer to attack successfully maize, sorghum (*Andropogon sorghum*) and Paddy (Gupta, 1937).

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*Melibiose = glucose-galactose.

**Raffinose = fructose-glucose-galactose

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Table 1. Digestive enzymes in salivary glands and various gut regions of Sesamia inferens.

Enzyme tested	Salivary glands	Foregut T	Foregut C	Midgut T	Midgut C	Hindgut T	Hindgut C
I. Carbohydrases							
1. Amylase (3.2.1.1)	-	-	-	?	?	-	-
2. Cellulase (3.2.1.4)	-	-	-	-	-	-	-
3. α-glucosidases							
i. Maltase (3.2.1.20)	±	-	±	+	++	-	-
ii. Melezitase	-	-	-	+	++	-	-
iii. Sucrase (3.2.1.26)	+++	-	++	-	+++	-	+
iv. Trehalase (3.2.1.28)	++	+	++	++	+++	+	+
4. β-glucosidase (3.2.1.21)	-	-	-	+	++	-	+
5. α-galactosidase (3.2.1.22)	-	-	-	-	-	-	-
6. β-galactosidase (3.2.1.23)	-	-	-	+	++	-	-
7. β-fructosidase (3.2.1.80)	++	-	±	-	++	-	-
II. Proteases							
1. Trypsin (3.4.4.4)	-	-	-	+	++	±	±
2. Aminopeptidases							
i. Aminotripeptidase (3.4.1.3)	++	-	+	+	+++	-	+++
ii. Leucine aminopeptidase (3.4.1.1)	+	-	+	++	++	+	++
3. Carboxypeptidase (3.4.2.1)	-	-	-	-	-	-	-
4. Dipeptidases							
i. Glycyl-glycine dipeptidase (3.4.3.1)	-	-	-	+	++	-	+
ii. Glycyl-L-leucine dipeptidase (3.4.3.2)	++	-	±	-	++	-	++
iii. Prolinase (3.4.3.6)	+	-	+	+	+++	+	++
iv. Prolidase (3.4.3.7)	-	-	-	-	-	-	-

T = Tissue; C = Contents; - Activity absent; ? Sometimes very weak activity; ± Very weak activity; + Weak activity; ++ Moderate activity and +++ High activity.

Table 2. Hydrolyzing capacity of S. inferens α-glucosidases of salivary glands and different gut regions.

Enzyme source	α-glucoside hydrolyzed	α-glucoside not hydrolyzed
Salivary glands	Maltose, sucrose and trehalose.	Melezitose.
Foregut tissue	Trehalose.	Maltose, melezitose and sucrose.
Foregut contents	Maltose, sucrose and trehalose.	Melezitose.
Midgut tissue	Maltose, melezitose and trehalose.	Sucrose.
Midgut contents	Maltose, melezitose, sucrose and trehalose.	
Hindgut tissue	Trehalose.	Maltose, melezitose and sucrose.
Hindgut contents	Sucrose and trehalose.	Maltose and melezitose.